U.S. Application No.	International Application No. PCT/JP00/01801	Attorney Docket No. SAEGU92.001APC

Date: September 20, 2001

09/937365 Page 1

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.:	PCT/JP00/01801
International Filing Date:	March 24, 2000
Priority Date Claimed:	June 21, 1999; April 30, 1999; March 26, 1999
Title of Invention:	COMPOSITIONS FOR PREVENTING AND TREATING TYPE I ALLERGY
Applicant(s) for DO/EO/US:	Mayumi Kotani, Akihito Fujita, Motonobu Matsumoto

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.

2. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

3. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

4. (X) A copy of the International Application as filed (35 USC 371(c)(2))

   a) () is transmitted herewith (required only if not transmitted by the International Bureau).
   b) () has been transmitted by the International Bureau.
   c) (X) a copy of Form PCT/1B/308 is enclosed.
   d) () is not required, as the application was filed in the United States Receiving Office (RO/US).

5. (X) Verification of translation.

6. (X) PCT Request with a translation of the International Application into English (35 USC 371(c)(2)).

7. (X) 2 sheets of drawings.

8. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))

   a) () are transmitted herewith (required only if not transmitted by the International Bureau).
   b) () have been transmitted by the International Bureau.
   c) () have not been made; however, the time limit for making such amendments has NOT expired.
   d) (X) have not been made and will not be made.

9. (X) A copy of the International Preliminary Examination Report with any annexes thereto, such as any amendments made under PCT Article 34.

10. (X) A FIRST preliminary amendment.

11. (X) Cover sheet for International Application as published.

12. (X) International Search Report.

KNOBBE, MARTENS, OLSON & BEAR, LLP
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(949) 760-0404 FAX (949) 760-9502

13. (X) A return prepaid postcard.

14. (X) The following fees are submitted:

				FEES
	BASIC FEE			$860
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**	
Total Claims	18 - 20 =	0 ×	$18	$0
Independent Claims	3 - 3 =	0 ×	$80	$0
	TOTAL OF ABOVE CALCULATIONS		$860	
	TOTAL FEES ENCLOSED			$860

15. (X) The fee for later submission of the signed oath or declaration set forth in 37 CFR 1.492(e) will be paid upon submission of the declaration.

16. (X) A check in the amount of $860 to cover the above fees is enclosed.

17. (X) The Commissioner is hereby authorized to charge only those additional fees which may be required, now or in the future, to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Daniel E. Altman
Reg. No. 34,115
Customer No. 20,995

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I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on

December 17, 2001
(Date)

Jennifer A. Haynes, Ph. D., Reg. No. 48,868

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.: PCT/JP00/01801
International Filing Date: March 24, 2000
Priority Date Claimed: March 26, 1999; April 30, 1999; June 21, 1999
Title of Invention: COMPOSITIONS FOR PREVENTING AND TREATING TYPE I ALLERGY
Applicant(s) for DO/EO/US: Mayumi Kotani, Akihito Fujita, Motonobu Matsumoto

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

(X) This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

(X) Copy of Notification of Missing Requirements Under 35 U.S.C. 371 In The United States Designated/Elected Office (DO/EO/US) dated November 1, 2001.

(X) An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

(X) A return prepaid postcard.

(X) The fee of $130 for submission of the Declaration after 30 months from the priority under 37 C.F.R. 1.492(e).

(X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 11-1410.

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By: Jennifer Haynes
Jennifer A. Haynes, Ph. D.
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09/937365

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Jennifer A. Haynes, Ph.D.
Patent Scientist

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

Attorney Docket No.	:	SAEGU92.001APC
Applicant(s)	:	Mayumi et al.
For	:	COMPOSITIONS FOR PREVENTING AND TREATING TYPE I ALLERGY
Attorney	:	Daniel E. Altman
"Express Mail" Mailing Label No.	:	EL672820960US
Date of Deposit	:	September 21, 2001

I hereby certify that the accompanying

Transmittal letter; PCT Form 308; Verification of A Translation; PCT Request Form with translation of the Int'l. Application into English; 2 sheets of drawings; IPER; Preliminary Amendment; Cover Sheet for Int'l. Application as published; Int'l. Search Report; Check for Filing Fee; Return Prepaid Postcard

are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and are addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Jose Colunga

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092001

SAEGU92.001APC

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Mayumi, et al.	Group Art Unit Unknown
Int'l Appl. No.	:	PCT/JP00/01801	
Int'l Filing Date	:	March 24, 2000	
For	:	COMPOSITIONS FOR PREVENTING AND TREATING TYPE I ALLERGY	
Examiner	:	Unknown	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to Examination on the merits, please amend the above-captioned International Application as follows:

IN THE SPECIFICATION

On page 1, line 2, after the Title of the Invention, please insert:

--This is the U.S. National Phase under 35 U.S.C.§371 of International Application PCT/JP00/01801, filed March 24, 2000, which claims priority of Japanese Applications JP 1999-84395, filed March 26, 1999, JP 1999-123633, filed April 30, 1999, and JP 1999-173731, filed June 21, 1999 (all of which are herein incorporated by reference).--.

On page 2, please replace the first paragraph (starting on line 4 and ending on line 9) as follows:

--An object of the present invention is to provide a food composition, a pharmaceutical composition and an external preparation for skin, which comprises as an active ingredient a compound surprising effective at suppressing type I allergy and its symptoms, and thus having an excellent preventative or therapeutic effect on type I allergy .--.

On page 2, please replace the third paragraph (starting on line 15 and ending on line 19) as follows:

--The inventors discovered, during atopic dermatitis screening, that astragalin is capable of suppressing atopic dermatitis and can also suppress a rise in serum IgE level, and also discovered that astragalin suppresses the symptoms of pollinosis.--.

On page 4, please replace the Figure legends (starting on line 7 and ending on line 11) as follows:

-- Fig. 1 shows the suppressive effect of kaempferol-3-glucoside (astragalin) on passive cutaneous anaphylaxis (PCA) in mice (Experimental Example 1);

Fig. 2 shows the suppressive effect of astragalin on histamine release (Experimental Example 2);--.

On page 5, please replace the third paragraph (starting on line 13 and ending on line 16) as follows:

--Astragalin is capable of suppressing a rise in serum IgE level, and hence the food composition of the present invention can also be used for suppressing a rise in serum IgE level.--.

On page 14, please replace the first paragraph (starting on line 3 and ending on line 6) as follows:

--Astragalin is capable of suppressing a rise in serum IgE level, and hence the pharmaceutical composition of the present invention can also be used for suppressing a rise in serum IgE level.--.

On page 17, please replace the second paragraph (starting on line 9 and ending on line 11) as follows:

--The external preparation for skin of the present invention is capable of improving rough skin conditions, and hence can be used for improving rough skin conditions.--.

On page 30, please replace the first paragraph (starting on line 1 and ending on line 6) with the following:

--Two of the volunteers had a rough skin condition before treatment in the form of administration of the astragalin solution by drinking, but the condition improved while drinking the astragalin solution. Rough skin conditions can also be expected to be improved upon applying astragalin to the skin in the form of a cosmetic.--.

On page 33, please cancel the word "CLAIMS" and substitute in its place --WHAT IS CLAIMED IS:--.

IN THE CLAIMS

Please cancel Claims 2-6.

Please replace the remaining claims with the following claims:

1. **(Amended)** A composition for preventing or treating type I allergy and diseases associated with type I allergy, comprising kaempferol-3-glucoside (astragalin) in an amount effective to prevent or treat type I allergy and the diseases associated with type I allergy.

7. **(Amended)** A method for preventing or treating type I allergy and diseases associated with type I allergy in a mammal,comprising:

administering to said mammal an effective amount of kaempferol-3-glucoside to prevent or treat type I allergy and the diseases associated with type I allergy.

8. **(Amended)** The method according to claim 7, wherein the diseases associated with type I allergy are atopic diseases.

9. **(Amended)** The method according to claim 8, wherein the diseases associated with type I allergy are selected from the group consisting of: atopic dermatitis, brochial asthma, allergic rhinitis, allergic contact dermatitis, pollinosis, and urticaria.

Please add the following claims:

10. The method according to claim 7 wherein the effective amount is from about 0.025 to about 3 mg per day per kg of body weight.

11. The method of claim 10 wherein the effective amount is from about 0.05 to about 1.5 mg per day per kg of body weight.

12. The method of claim 7 wherein the administration is selected from the group consisting of: orally, intravenously, topically, intramuscularly, intracutaneously, subcutaneously, intraperitoneally, and by aerosolization.

13. The method of claim 12, wherein the administration is orally, admixed with a food product.

14. The method of claim 13, wherein the food product is selected from the group consisting of: juice, soft drinks, teas, powdered soups, jelly, cookies, biscuits, cereal, crackers, candy, breads, noodles, fish paste, chewing gum, ice cream, and chocolate.

15. The method of claim 7 wherein the administration is between one and 4 doses per day.

16. The composition according to claim 1 wherein the effective amount is from about 0.025 to about 3 mg per day per kg of body weight.

17. The composition of claim 1 wherein the effective amount is from about 0.05 to about 1.5 mg per day per kg of body weight.

18. A pharmaceutical composition comprising the composition of claim 1 with a pharmaceutically acceptable carrier, diluent, or excipient.

19. The pharmaceutical composition of claim 18, wherein said carrier, diluent, or excipient is selected from the group consisting of: powders, lotions, ointments, binders, surfactants, moisturizers, fillers, extenders, wetting agents and food products.

20. The pharmaceutical composition of claim 18 further comprising: antiseptics, colerants, preservatives, antioxidants, aromatics, and food products.

21. The composition of claim 1 wherein said kaempferol-3-glucoside is extracted from plants or chemically synthesized.

22. The composition of claim 21, wherein said plants are selected from the group consisting of: persimmon, amachazuru, gymnema, guava, kuko, striped bamboo, jasmine, sugina, dokudami, loquat, sen-cha, and tien-cha.

23. A method for the reduction of serum IgE in a mammal, comprising:

administering an amount of kaempferol-3-glucoside to said mammal sufficient to reduce serum IgE.

REMARKS

The claims and specification have been amended to correct minor deficiencies and inconsistencies. No new matter has been added herewith. Support for amended claim 9 can be found in the Specification, page 5, lines 5-10. Support for claims 12-14 can be found in the Specification, page 10, lines 17 through page 11, line 7. Support for claims 16-17 can be found in the Specification, page 12, lines 1-5. Support for added claims Support for added claims 21 and 22 can be found in the specification on page 5, line 23 through page 6, line 12. Support for Claim 23 can be found on page 5, line 13-16.

The changes made to the claims by the current amendment, including **[deletions]** and additions are shown on an attached sheet entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**, which follows the signature page of this Amendment.

Conclusion

Should there be any questions relating to the above-captioned patent application, the Examiner is respectfully requested to contact the undersigned at the telephone number appearing

COMPOSITIONS FOR PREVENTING AND TREATING TYPE I ALLERGY

CLAIMS AS FILED

Please cancel Claims 2-6.

Please replace the remaining claims with the following claims:

1. **(Amended)** A composition for preventing or treating type I allergy and diseases associated with type I allergy, comprising kaempferol-3-glucoside (astragalin) in an amount effective to prevent or treat type I allergy and the diseases associated with type I allergy.

7. **(Amended)** A method for preventing or treating type I allergy and diseases associated with type I allergy in a mammal,comprising:

administering to said mammal an effective amount of kaempferol-3-glucoside to prevent or treat type I allergy and the diseases associated with type I allergy.

8. **(Amended)** The method according to claim 7, wherein the diseases associated with type I allergy are atopic diseases.

9. **(Amended)** The method according to claim 8, wherein the diseases associated with type I allergy are selected from the group consisting of: atopic dermatitis, brochial asthma, allergic rhinitis, allergic contact dermatitis, pollinosis, and urticaria.

Please add the following claims:

10. The method according to claim 7 wherein the effective amount is from about 0.025 to about 3 mg per day per kg of body weight.

11. The method of claim 10 wherein the effective amount is from about 0.05 to about 1.5 mg per day per kg of body weight.

12. The method of claim 7 wherein the administration is selected from the group consisting of: orally, intravenously, topically, intramuscularly, intracutaneously, subcutaneously, intraperitoneally, and by aerosolization.

13. The method of claim 12, wherein the administration is orally, admixed with a food product.

14. The method of claim 13, wherein the food product is selected from the group consisting of: juice, soft drinks, teas, powdered soups, jelly, cookies, biscuits, cereal, crackers, candy, breads, noodles, fish paste, chewing gum, ice cream, and chocolate.

15. The method of claim 7 wherein the administration is between one and 4 doses per day.

16. The composition according to claim 1 wherein the effective amount is from about 0.025 to about 3 mg per day per kg of body weight.

17. The composition of claim 1 wherein the effective amount is from about 0.05 to about 1.5 mg per day per kg of body weight.

18. A pharmaceutical composition comprising the composition of claim 1 with a pharmaceutically acceptable carrier, diluent, or excipient.

19. The pharmaceutical composition of claim 18, wherein said carrier, diluent, or excipient is selected from the group consisting of: powders, lotions, ointments, binders, surfactants, moisturizers, fillers, extenders, wetting agents and food products.

20. The pharmaceutical composition of claim 18 further comprising: antiseptics, colerants, preservatives, antioxidants, aromatics, and food products.

21. The composition of claim 1 wherein said kaempferol-3-glucoside is extracted from plants or chemically synthesized.

22. The composition of claim 21, wherein said plants are selected from the group consisting of: persimmon, amachazuru, gymnema, guava, kuko, striped bamboo, jasmine, sugina, dokudami, loquat, sen-cha, and tien-cha.

23. A method for the reduction of serum IgE in a mammal, comprising:

administering an amount of kaempferol-3-glucoside to said mammal sufficient to reduce serum IgE.

below. Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 21 Sept. 2001

By: Daniel Altman

Daniel E. Altman
Registration No. 34,115
Attorney of Record
620 Newport Center Drive
Sixteenth Floor
Newport Beach, CA 92660

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

On page 1, line 2, after the Title of the Invention, please insert:

--This is the U.S. National Phase under 35 U.S.C.§371 of International Application PCT/JP00/01801, filed March 24, 2000, which claims priority of Japanese Applications JP 1999-84395, filed March 26, 1999, JP 1999-123633, filed April 30, 1999, and JP 1999-173731, filed June 21, 1999 (all of which are herein incorporated by reference).--.

On page 2, please replace the first paragraph (starting on line 4 and ending on line 9) as follows:

--An object of the present invention is to provide a food composition, a pharmaceutical composition and an external preparation for skin, which comprises as an active ingredient a compound **[having excellent]**surprising effective at suppressing type I allergy **[suppression effect]**and its symptoms, and thus **[have]**having an excellent preventative or therapeutic effect on type I allergy **[preventive or therapeutic effect]**.--.

On page 2, please replace the third paragraph (starting on line 15 and ending on line 19) as follows:

--The inventors discovered, during atopic dermatitis screening, that astragalin **[has an action]**is capable of suppressing atopic dermatitis and **[an action of suppressing]**can also suppress a rise in serum IgE level, and also discovered that astragalin suppresses the symptoms of pollinosis.--.

On page 4, please replace the Figure legends (starting on line 7 and ending on line 11) as follows:

-- Fig. 1 shows the suppressive effect of kaempferol-3-glucoside (astragalin) **[of suppressing]**on passive cutaneous anaphylaxis (PCA) in mice (Experimental Example 1);

Fig. 2 shows the suppressive effect of astragalin **[of suppressing]**on histamine release (Experimental Example 2);--.

On page 5, please replace the third paragraph (starting on line 13 and ending on line 16) as follows:

--Astragalin **[has an action]**is capable of suppressing a rise in serum IgE level, and hence the food composition of the present invention can also be used for suppressing a rise in serum IgE level.--.

On page 14, please replace the first paragraph (starting on line 3 and ending on line 6) as follows:

--Astragalin **[has an action]**is capable of suppressing a rise in serum IgE level, and hence the pharmaceutical composition of the present invention can also be used for suppressing a rise in serum IgE level.--.

On page 17, please replace the second paragraph (starting on line 9 and ending on line 11) as follows:

--The external preparation for skin of the present invention **[has an action]**is capable of improving rough skin conditions, and hence can be used for improving rough skin conditions.--.

On page 30, please replace the first paragraph (starting on line 1 and ending on line 6) with the following:

--Two of the volunteers had a rough skin condition **[at the time of starting to drink]**before treatment in the form of administration of the astragalin solution by drinking, but the condition improved while drinking the astragalin solution. Rough skin conditions can also be expected to be improved upon applying astragalin to the skin in the form of a cosmetic.--.

On page 33, please cancel the word "CLAIMS" and substitute in its place --WHAT IS CLAIMED IS:--.

IN THE CLAIMS

Please cancel Claims 2-6.

Please replace the remaining claims with the following claims:

1. **(Amended)** A composition for preventing or treating type I allergy and diseases associated with type I allergy, comprising kaempferol-3-glucoside (astragalin) **[as an active ingredient]**in an amount effective to prevent or treat type I allergy and the diseases associated with type I allergy.

7. **(Amended)** A method for preventing or treating type I allergy and diseases associated with type I allergy in a mammal,**[by ingesting or]**comprising:

administering to said mammal an effective amount of kaempferol-3-glucoside to prevent or treat type I allergy and the diseases associated with type I allergy.

8. **(Amended)** The method according to claim 7, wherein the diseases associated with type I allergy are atopic diseases.

9. **(Amended)** The method according to claim **[**7**]**8, wherein the diseases associated with type I allergy **[is pollinosis]**are selected from the group consisting of: atopic dermatitis, brochial asthma, allergic rhinitis, allergic contact dermatitis, pollinosis, and urticaria.

Please add the following claims:

10. The method according to claim 7 wherein the effective amount is from about 0.025 to about 3 mg per day per kg of body weight.

11. The method of claim 10 wherein the effective amount is from about 0.05 to about 1.5 mg per day per kg of body weight.

12. The method of claim 7 wherein the administration is selected from the group consisting of: orally, intravenously, topically, intramuscularly, intracutaneously, subcutaneously, intraperitoneally, and by aerosolization.

13. The method of claim 12, wherein the administration is orally, admixed with a food product.

14. The method of claim 13, wherein the food product is selected from the group consisting of: juice, soft drinks, teas, powdered soups, jelly, cookies, biscuits, cereal, crackers, candy, breads, noodles, fish paste, chewing gum, ice cream, and chocolate.

15. The method of claim 7 wherein the administration is between one and 4 doses per day.

16. The composition according to claim 1 wherein the effective amount is from about 0.025 to about 3 mg per day per kg of body weight.

17. The composition of claim 1 wherein the effective amount is from about 0.05 to about 1.5 mg per day per kg of body weight.

18. A pharmaceutical composition comprising the composition of claim 1 with a pharmaceutically acceptable carrier, diluent, or excipient.

19. The pharmaceutical composition of claim 18, wherein said carrier, diluent, or excipient is selected from the group consisting of: powders, lotions, ointments, binders, surfactants, moisturizers, fillers, extenders, wetting agents and food products.

20. The pharmaceutical composition of claim 18 further comprising: antiseptics, colerants, preservatives, antioxidants, aromatics, and food products.

21. The composition of claim 1 wherein said kaempferol-3-glucoside is extracted from plants or chemically synthesized.

22. The composition of claim 21, wherein said plants are selected from the group consisting of: persimmon, amachazuru, gymnema, guava, kuko, striped bamboo, jasmine, sugina, dokudami, loquat, sen-cha, and tien-cha.

23. A method for the reduction of serum IgE in a mammal, comprising:

administering an amount of kaempferol-3-glucoside to said mammal sufficient to reduce serum IgE.

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below:

That I am knowledgeable in the English language and in the language in which the below identified international application was filed, and that I believe the English translation of the international application No. PCT/JP00/01801 is a true and complete translation of the above identified international application as filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Date

September 10, 2001

Full name of the translator Ikuko AIHARA

Signature of the translator Ikuko Aihara

Post Office Address Kitahama TNK Building 7-1, Dosho-machi 1-chome, Chuo-ku, Osaka-shi, Osaka 541-0045 Japan

DESCRIPTION

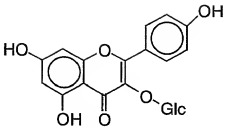
COMPOSITIONS FOR PREVENTING AND TREATING TYPE I ALLERGY

TECHNICAL FIELD

The present invention relates to compositions comprising kaempferol-3-glucoside for preventing or treating type I allergy, and more particularly to a food composition for preventing type I allergy, a pharmaceutical composition for preventing or treating type I allergy and an external preparation for skin for preventing or treating type I allergy.

BACKGROUND ART

In recent years it has been reported that various substances contained in plants have antiallergic actions. For example, it has been reported that kaempferol, which is a type of flavonoid, has type I allergy suppression effect. However, this effect is not sufficient. The type I allergy suppression effect of kaempferol-3-glucoside (also referred to as 'astragalin'), which is a glycoside of kaempferol and is represented by undermentioned general formula (1), on the other hand, has not previously been found.



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DISCLOSURE OF INVENTION

An object of the present invention is to provide a food composition, a pharmaceutical composition and an external preparation for skin, which comprise as an active ingredient a compound having excellent type I allergy suppression effect, and thus have type I allergy preventive or therapeutic effect.

The inventors found, during type I allergy screening, that kaempferol-3-glucoside (astragalin) has an excellent action of suppressing passive cutaneous anaphylaxis in mice. The present invention was accomplished based on this finding.

The inventors discovered, during atopic dermatitis screening, that astragalin has an action of suppressing atopic dermatitis and an action of suppressing a rise in serum IgE level, and also discovered that astragalin suppresses the symptoms of pollinosis.

The present invention thus provides the items listed

below:

Item 1. A composition for preventing or treating type I allergy and diseases associated with type I allergy, comprising kaempferol-3-glucoside as an active ingredient.

Item 2. The composition according to item 1, wherein the composition is a food composition for preventing type I allergy and diseases associated with type I allergy.

Item 3. The composition according to item 1, wherein the composition is a pharmaceutical composition for preventing or treating type I allergy and diseases associated with type I allergy.

Item 4. The composition according to item 1, wherein the composition is an external preparation for skin for preventing or treating type I allergy and diseases associated with type I allergy.

Item 5. The composition according to item 1, wherein the diseases associated with type I allergy are atopic diseases.

Item 6. The composition according to item 1, wherein the disease associated with type I allergy is pollinosis.

Item 7. A method for preventing or treating type I allergy and diseases associated with type I allergy by ingesting or administering an effective amount of kaempferol-3-glucoside.

Item 8. The method according to item 7, wherein the

diseases associated with type I allergy are atopic diseases.

Item 9. The method according to item 7, wherein the disease associated with type I allergy is pollinosis.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows effect of kaempferol-3-glucoside (astragalin) of suppressing passive cutaneous anaphylaxis (PCA) in mice (Experimental Example 1);

Fig. 2 shows effect of astragalin of suppressing histamine release (Experimental Example 2);

Fig. 3 shows changes over time in dermal symptoms in NC/Nga mice after administration of astragalin (Experimental Example 3); and

Fig. 4 shows changes over time in serum IgE level in NC/Nga mice after administration of astragalin (Experimental Example 4).

DETAILED DESCRIPTION OF THE INVENTION

A composition comprising kaempferol-3-glucoside for preventing or treating type I allergy according to the present invention can be used as a food composition, a pharmaceutical composition or an external preparation for skin.

1. Food composition

The food composition of the present invention can be used in the prevention of type I allergy and allergic diseases associated with type I allergy.

Examples of allergic diseases associated with type I allergy include atopic dermatitis, bronchial asthma, allergic rhinitis and other atopic diseases (sometimes referred to merely as 'atopy'), allergic contact dermatitis, pollinosis and urticaria. Of these, the food composition of the present invention is preferably used for preventing pollinosis and atopic diseases (in particular atopic dermatitis).

Astragalin has an action of suppressing a rise in serum IgE level, and hence the food composition of the present invention can also be used for suppressing a rise in serum IgE level.

The serum IgE level suppressing food of the present invention can be applied to all diseases accompanied by a rise in serum IgE level without limitation. Examples of diseases accompanied by a rise in serum IgE level include atopic dermatitis, bronchial asthma, allergic rhinitis, food allergies, pollinosis and urticaria.

The kaempferol-3-glucoside (astragalin) comprised in the food composition of the present invention can be synthesized by known methods. Moreover, astragalin is

contained in various plants, and hence plant-derived astragalin may also be used. In the case that a plant is the source, either the purified astragalin or an extract comprising astragalin may be used as the astragalin.

Preferable examples of plants containing large amounts of astragalin include persimmon (Diospyros kaki) leaves, *amachazuru* (Gynostemma pentaphylla), gymnema, guava (Psidium guajava), *kuko* (Lycium chinense), striped bamboo (Sasa veitchii), jasmine (Jasminum officinale), sugina (Equisetum arvense L.), *dokudami* (Houttuynia cordata), *hatomugi* (Coix mayuen Roman.), loquat (Eriobotrya japonica) leaves, sen-cha , and tien-cha.

Other examples of plants containing large amounts of astragalin include the following: Securigera securidacea (L.) Deg. et Dorfl. (Fabaceae) seed, Vahlia capensis, Moroheiya (Vietnamese Corchorus olitorius L. (Tiliaceae)), Alsophila spinulosa (Hook) Tryon., Camellia sinensis O. Kuntze, Ochradenus baccatus., Milkvetch root (Radix Astragali), Glycyrrhiza uralensis Ficsh (Leguminosae), zhongfeng naomai tong oral liquid, Mussaenda arcuata Lam. ex Poiret, Eupatorium cannabinum L., persimmon (Dispyros kaki), Wikstroemia indica, Dianthus barbatus cv. ('China Doll', Caryophyllaceae), Anodendron affine Durce., Coronilla varia L., Magnolia fargesii, Ailanthus altissima, Aralia continentalis kitagawa (Araliacene), Tribulus

terrestris Linn, Ochna obtusata (Ochnacene), Hedera helix L. (Araliaceae), Impatiens balsamina L., Circaea lutetiana ssp. Canadensis, Herniaria mauritanica Murbeck, Glycyrrhiza globra, Glycyrrhiza echinata, Glycyrrhiza pallidiflora, Glycyrrhiza foctida, Aconitum pseudolaeve var. erectum, saffron (Crocus sativus), Cucurbita pepo L., Pulmonaria officinalis, Potentilla anserina L. (Rosaceae), Phyllanthus emblica, Querucus pedunculata, Rumex cyprius, Terminalia bellerica, Terminalia chebula, Terminalia horrida, Corchorus olitorius L., Polygonum aviculare, Kummerowia striata, Morus alba L., Agrimonia eupatoria, Drosera rotundifolia L. (Droseraceae), Lysimachiae herba, Lysimachia chiristinae var. typica, and Scolymus hispanicus.

Other examples include the following plants: Euonymus species, Morus insignis, Pyrrosia lingua, Apoynum venetum L., Poacynum hendersonii (Hook f.) woodson, Hedyosmum bonplandianum, H.B.K. (Chloranthaceae), Carthamus tinctorius, Orostachys japonicus, Eucommia ulmoides, Polyganum cognatum, Erythroxylon myrsinites, Mussaenda arcuata, Escallonia illinita Presl., Helichrysum italicum G. Don (compositae), Artemisia annua L., Astragalus aitosensis, Eupatorium guayanum, Helichrysum species, Diplazium nipponieum Tagawa, Festuca Asgentina, Athaea officinalis, Tinospora malabarica Miers, Coronilla

varia L., Chinese tallow tree (Sapium sebiferum), fern Lygodium flexuosum, Asanthus, Helichrysum graveolens, Arabidopsis thaliana (L.) Heynh., Cleome droserifolia, Helichrysum sanguineum, Helichrysum noeanum Boiss. (Asteraceae), Epilobium fleischeri, Epilobium adenocaulon, Epilobium palustre, Astrantia major L., Hirschfeldia incana., Digitalis lanata, Quercus ilex L., Smyrnium perfoliatum, Smyrnium creticum, Smyrnium rotundifolium, Ascarina lucida, Helichrysum armenium, Maclura pomifera fruit, Castanea sativa Mill, Tussilago farfara L., Anchusa officinalis L., Cyathea contaminans Copel, Solidago virgaurea L. var. leiocarpa (Benth.) A., Helichrysum Plicatum DC. ssp. polyphyllum (Ledeb.) Davis-Kupicha, Choisya ternata Kunth, Pteridium aquilinum var. Latiusculum IV., and Isopyrum thalictroides L. II.

In addition to the above, the following plants also contain astragalin: Cassia obtusifolia L., Helichrysum plicatum DC, Convallaria maialis, Falcaria vulgaris Bernh. (Umbelliferae), Umckaloabo, Clitoria ternatea L., Larix needles, Helichrysum orientale (L.) Gaertner, Ageratum mexicanum Sims. (Compositae), Ribes nigrum, Mangifera indica, Synadenium carinatum, Papaver radicatum, Loropetalum Chinense, Scot pine (Pinus sylvestris L.), Cuscuta australis R. Br., Allium victorialis L., Sapium japonicum (Euphorbiaceae), Euphorbia pekinensis, Viburnum

awabuki, Ilex centrochinensis, Polygonum aviculare, Atractylodes lancea DC.(Composieae), carthami flos., Lonicera japonica, Glycyrrhiza uralensis Fisch., Althaea officinalis var. russalka, Alhagi persarum Boiss. and Buhse., Quercus-ilexl, Mulberry (Morus alba) leaves, Hippophae-phamnoides, Astragalus membranaceous Bge. var. mogholicus (Bge.) Hsiao, Fengrutong granule, Cirsium setosum, Analphalis contorta Hooker, beggarticks (Bidens parviflora), tormentil, Apocynum hendersonii Hook. F., Astragalus dipelta, Gliricidia sepium, Cyclachaena xanthifolia, Helichrysum noeanum Boiss. (Asteraceae), Persica vulgaris, Rhododendron micranthum Turcz, Viburnum urceolatum, Salix caprea, Salix alba, Orobus vernus, Lepidium draba, Lepidium ruderale, Onobrychis pulchella, Onobrychis tanaitica, Onobrychis arenaria, Asclepias incarnata, Orchis sambucina, Astragalus ammodendron, Syringa vulgaris leaves, Picea obovata needles, Osmunda japonica, Potentilla tanacerifolia, Astragalus flexus, Aesculus indica., Doronicum macrophyllum, Doronicum oblongifolium, Astragalus testiculatus, Pteridaceae, Onobrychis vassiltschenkoi, Fraxinus raibocarpa, Boehmeria tricuspis, Boehmeria holosericea, Komarov's oxytropis, Trifolium hybridum, Trifolium ambiguum, Delphinium, Campanula hypopolia, Homogyne, Pteridium aquilinum, Vaccinium myrtillus, Oxytropis lanata, Sempervivum

ruthenicum, Cucurbita maxima, Anodendron affine, Quercus pontica, Baccharis angustifoia, Berlandiera pumila, Padus avium, Onobrychis kachetica, Onobrychis inermis, Sempervivum ruthenicum, Lupinus luteus, Alcea nudiflora, Rhus coriaria, Gymnadenia conopea, Spiraea media, Adiantum capillus-veneris, Adiantum cuneatum, Corydalis lutea, Ononis arvensis, Paeonia arborea, Paeonia suffruticosa, Bauhinia purpurea, Sorbus pendula, Arnica species, and Nyctanthes arbor-tristis.

The astragalin content of the food composition of the present invention can be selected from a wide range without limitation, so long as the intended effects are obtained. The astragalin content is generally in a range of about 0.00001 to 80% relative to the total weight of the composition (here and hereinafter '%' means 'weight %'), preferably about 0.0001 to 70%.

The food composition of the present invention can be prepared by mixing astragalin into a carrier comprising food ingredients, additives and the like, and then following conventional methods for the food form to be made.

The food composition of the present invention can be prepared in any of various forms. Examples include liquid beverages such as juices, soft drinks and teas; powdered beverages such as powdered juices and powdered soups;

confectionery such as chocolates, candies, chewing gums, ice creams, jellies, cookies, biscuits, corn flakes, chewable tablets, gummi candies, wafers and rice crackers; seasonings such as dressings and sauces; breads; noodles; *konnyaku* (arum root paste); fish paste products such as *kamaboko*; and *furikake* (a seasoned powder for sprinkling on cooked rice).

The food composition of the present invention may comprise food ingredients and additives usually incorporated into foods of the form to be made. Examples of additives include sweeteners, colorants, antioxidants, vitamins and aromatics.

The food composition of the present invention may also comprise plants such as crude drugs and herbs (chamomile, ginger, rose hip etc.) or extracts thereof.

The food composition of the present invention can also be used as a food ingredient used in the preparation of any food. When the food composition of the present invention is used as such a food ingredient, it may be added to a food product that has already been prepared, for example a commercially available beverage.

The intake amount of the food composition of the present invention for preventing type I allergy is suitably selected in accordance with conditions such as the form of the food and the age and sex of the person

ingesting the food, but is generally such that the daily intake of astragalin per kg of body weight is in a range of about 0.025 to 3 mg, preferably about 0.05 to 1.5 mg. The food may be ingested either once per day or in 2 to 4 divided amounts per day.

The food composition of the present invention has a type I allergy preventive action, and can be used as a health food, a functional food, a nutritional supplement food, a food for specified health use, a food for sick persons, and so on.

For example, the food composition of the present invention may be ingested with the purpose of prevention by a person who is at risk of developing pollinosis, such as a person who has previously experienced pollinosis. In such a case, the food composition may be ingested throughout the year, but is preferably ingested starting a few weeks before the start of the pollen season.

Moreover, the food composition of the present invention may be ingested with the purpose of preventing atopy by, for example, a person who has previously experienced atopy, a person predisposed to allergies, an infant or the like.

The food composition of the present invention can also be used as a livestock feed or a pet food. The food composition may be in any form conventionally used for

livestock feeds or pet foods. The food composition can be prepared by mixing astragalin together with food ingredients and additives usually incorporated into livestock feeds or pet foods, and then following conventional methods for the form to be made.

Provided the intended effects of the present invention are obtained, the astragalin content and the intake amount of the livestock feed or pet food can be selected without limitation in accordance with the form thereof, the type of livestock or pet, and so on, and referring to the case of a food composition for human consumption described above.

2. Pharmaceutical composition

The pharmaceutical composition of the present invention can be used in the prevention or treatment of type I allergy and allergic diseases associated with type I allergy.

Examples of allergic diseases associated with type I allergy include atopic dermatitis, bronchial asthma, allergic rhinitis and other atopic diseases (sometimes referred to merely as 'atopy'), allergic contact dermatitis, pollinosis and urticaria. Of these, the pharmaceutical composition of the present invention is preferably used as an agent for preventing or treating

pollinosis and atopic diseases (in particular atopic dermatitis).

Astragalin has an action of suppressing a rise in serum IgE level, and hence the pharmaceutical composition of the present invention can also be used for suppressing a rise in serum IgE level.

The serum IgE level suppressant of the present invention can be applied to all diseases accompanied by a rise in serum IgE level without limitation. Examples of diseases accompanied by a rise in serum IgE level include atopic dermatitis, bronchial asthma, allergic rhinitis, food allergies, pollinosis and urticaria.

The astragalin comprised in the pharmaceutical composition of the present invention may be synthesized, or may be from an astragalin-containing plant.

The pharmaceutical composition of the present invention comprises astragalin as an essential component together with suitable pharmaceutically acceptable carriers, and is used in the form of a usual pharmaceutical form.

The unit dosage form of the pharmaceutical composition can be selected from various forms in accordance with the therapeutic purpose. Typical examples include solid preparations such as tablets, pills, granules, capsules and troches; powdered preparations such

as powders for internal use and powders for external use; liquid preparations such as solutions, suspensions, emulsions, injections (liquids, suspensions etc.), syrups, lotions, aerosols and ophthalmic solutions; cream-like preparations such as ointments; and cataplasms.

Examples of pharmaceutically acceptable carriers that may be used in the pharmaceutical composition of the present invention include binders, disintegrators, surfactants, absorption promoters, moisturizers, adsorbents, lubricants, fillers, extenders, humectants, and other diluents and excipients. Such carriers are selected in accordance with the unit dosage form to be obtained.

Moreover, if necessary, antiseptics, sweeteners, colorants, antioxidants, preservatives, aromatics, flavors and the like, and other medicines, can be incorporated into the pharmaceutical composition of the present invention during preparation.

The pharmaceutical composition can be prepared following conventional methods for the form to be made.

There are no particular limitations on the method of administering the pharmaceutical composition. The pharmaceutical composition is administered in accordance with the form thereof, for example orally in the case of tablets, pills, granules, capsules, troches, powders for

internal use, solutions, suspensions, emulsions and syrups, and percutaneously in the case of powders for external use, lotions, ointments and cataplasms. An injection can be administered intravenously, intramuscularly, intracutaneously, subcutaneously or intraperitoneally. An aerosol can be administered nasally as a collunarium.

Another possible administration method is for a patient to wear a mask prepared, for example, by immersing a gauze mask in a liquid preparation or coating or spraying a gauze mask with a liquid preparation, and then drying. Such a mask is preferably worn for the purpose of preventing or treating pollinosis, especially during the pollen season. Yet another possible administration method is for a patient to wear rubber gloves or the like that have had a powdered preparation applied onto the inside thereof in advance.

The dosage of the pharmaceutical composition is suitably selected in accordance with conditions such as the form of the preparation, the age and sex of the patient and the severity of the disease, but is generally such that the daily intake of astragalin per kg of body weight is in a range of about 0.025 to 3 mg, preferably about 0.05 to 1.5 mg. The pharmaceutical composition may be administered either once per day or in 2 to 4 divided doses per day.

When the pharmaceutical composition of the present invention is used for preventing or treating pollinosis, it can, for example, be administered with the purpose of prevention to a person who has previously experienced pollinosis, starting a few weeks before the start of the pollen season.

3. External preparation for skin

The external preparation for skin of the present invention has an action of improving rough skin conditions, and hence can be used for improving rough skin conditions.

The external preparation for skin of the present invention can be used for preventing or treating skin diseases associated with type I allergy such as atopic dermatitis, allergic contact dermatitis and urticaria, and rough skin conditions accompanying such diseases.

The astragalin mixed into the external preparation for skin of the present invention may be synthesized, or may be from an astragalin-containing plant.

The astragalin content of the external preparation for skin of the present invention can be suitably selected from a wide range without limitation, so long as the intended effects are obtained. The astragalin content is preferablly in a range of about 0.00001 to 80 % relative to the total weight of the preparation, more preferably

0.0001 to about 70 %.

The form of the external preparation for skin can be selected from various forms in accordance with the purpose. Specific examples include cleaning agents such as soaps, facial cleansers and shampoos, milky lotions, creams, emulsified preparations, ointments, other lotions, and preparations for use in the bath.

The external preparation for skin of the present invention comprises astragalin as an essential component, used together with suitable carriers in a conventional form for external preparation for skins.

Carriers used in the external preparation for skin of the present invention can be suitably selected from commonly used carriers in accordance with the form of the preparation. Such carriers include binders, surfactants, moisturizers, fillers, extenders, wetting agents, and other diluents and excipients.

Moreover, if necessary, antiseptics, colorants, preservatives, antioxidants, aromatics, and the like can be incorporated into the external preparation for skin of the present invention.

Moreover, crude drugs or herbs commonly mixed into external preparation for skins such as aloe, *dokudami* (Houttuynia cordata) and mugwort (Artemisia vulgaris) may also be mixed into the external preparation for skin of

the present invention.

The external preparation for skin of the present invention can be prepared following conventional methods for the form to be made.

There are no particular limitations on the amount used of the external preparation for skin of the present invention, so long as the intended effects are obtained. A suitable amount as determined by the form of the preparation, the condition of the skin, the degree of skin roughness and so on may be applied to the skin once a day, or 2 to 4 times a day.

The external preparation for skin of the present invention may be used not only when the skin is already rough, but also to prevent rough skin by people prone to rough skin such as people with sensitive skin.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention will now be described in more detail through experimental examples and formulation examples. However, the scope of the invention is not limited to only these examples.

The experimental setup used in undermentioned Experimental Example 1 is a typical setup used for type I allergy screening. The action of astragalin of

suppressing type I allergy was tested using this setup.

Experimental Example 1: Suppression of passive cutaneous anaphylaxis (PCA) in mice

Ten 5-week-old ddY male mice were purchased from Japan SLC, and were reared at a room temperature of 23 ± 3°C and a humidity of 55 ± 15%, with a 12 hour light-dark cycle (light period 7:00 to 19:00). The mice were kept 5 to a cage, and were fed a standard diet (Labo MR Stock, Nihon Nosan Kogyo K.K.) for a 7-day preliminary period, before being divided into a group to be administered astragalin and a control group each of 5 mice. A 0.025 % (w/v) solution of astragalin in distilled water was forcedly orally administered (1.25 mg/5 ml/kg) to the astragalin-administered group using a metal stomach tube, while distilled water was forcedly orally administered (5 ml/kg) to the control group. One hour after the administration, 20 µl of an anti-DNP mouse IgE antibody (10 µg/ml) was intracutaneously injected into the right auricula and 20 µl of physiological saline into the left auricula of each mouse. 24 hours after the intracutaneous injections, 100 µl of DNP-BSA (1 mg/ml) was intravenously injected into the tail of each mouse. 15 minutes later, the thicknesses of the left and right auriculae of each mouse were measured three times using a thickness gauge (Ozaki Seisakusho K.K.). The auricula swelling rate was

then calculated for each group using undermentioned equation 1, and the auricula swelling suppression rate was calculated using undermentioned equation 2. The measurement values were represented by the mean and standard deviation.

Auricula swelling rate (%) =

$$\frac{\text{Right auricula thickness} - \text{Left auricula thickness}}{\text{Left auricula thickness}} \times 100 \quad \text{(Eqn.1)}$$

Auricula swelling suppression rate (%) =

$$100 - \frac{\text{Mean auricula swelling rate for astragalin-administered group}}{\text{Mean auricula swelling rate for control group}} \times 100 \quad \text{(Eqn.2)}$$

For the control group administered distilled water, the left auricula thickness was 0.270 ± 0.017 mm, whereas the right auricula thickness had increased to 0.343 ± 0.040 mm, giving an auricula swelling rate of 27.2 ± 12.5 %; for the astragalin-administered group, on the other hand, the left auricula thickness was 0.242 ± 0.013 mm, whereas the right auricula thickness had increased to 0.286 ± 0.017 mm, giving an auricula swelling rate of 18.2

±5.0% (see Fig. 1). Auricula swelling was thus suppressed in the astragalin-administered group compared with in the control group, with the auricula swelling suppression rate being 33 %.

Through Experimental Example 1, it was thus verified that astragalin has an action of suppressing type I allergy.

A type I allergic reaction is accompanied by release of chemical transmitters such as histamine from the sensitized mastocytes or basocytes. After verifying that astragalin has an action of suppressing type I allergy through Experimental Example 1, the present inventors thus conducted the following test to find out whether or not astragalin has an effect of suppressing histamine release.

Experimental Example 2: Histamine release suppression test using human whole blood

Human whole blood was collected from healthy volunteers and heparin was added thereto. A blood sample was prepared by adding 6 parts by weight of a histamine release buffer (Immunotech) to 1 part by weight of the whole blood to which the heparin had been added. 200 µl of the blood sample and 100µl of a histamine release buffer comprising astragalin (33 µM), kaempferol (33 µM)

or epinephrine (1638 μM or 4917 μM) were placed in an Eppendorf tube and allowed to stand for 30 minutes while cooling in ice (final concentration: 11μM for astragalin, 11 μM for kaempferol, 546 μM or 1639μM for epinephrine).

Centrifugal separation (3000 rpm, 5 minutes, 4°C) was then carried out and the supernatant removed, and the resulting cells were again put into 300 μl of a histamine release buffer. 4.5 μl of 1 mg/ml CRA-1 (an anti human FcεRI receptor antibody, Cosmo Bio) was next added to the mixture (final CRA-1 concentration 15 μg/ml), and incubation was carried out for 30 minutes at 37°C. After centrifugation (3000 rpm, 10 minutes, 4°C), the amount of histamine in the supernatant was measured using a histamine EIA kit (Immunotech). The histamine release suppression rate (%) was then calculated using undermentioned equation 3 from histamine amounts calculated from a calibration curve. The measurements were carried out with N=3, and the measurement values were represented by the mean and standard deviation.

Histamine release suppression rate (%) =

$$100 - \frac{A-B}{C-B} \times 100 \quad \text{(Eqn.3)}$$

A: Amount of histamine released from cells to which astragalin/kaempferol/epinephrine added (n mole)

B: Amount of histamine released from untreated cells (n mole)

C: Amount of histamine released from cells to which only CRA-1 added (n mole)

The histamine release suppression results for astragalin, kaempferol and epinephrine are shown in Fig. 2. The histamine release suppression rate was 50 ± 7 % for astragalin (11 μM), and 31 ± 9% for kaempferol (11 μM). The histamine release suppression rate for epinephrine, which is a medicine, was 31 ± 3% at 546 μM and 55 ± 4% at 1639 μM. It can thus be seen that astragalin (kaempferol-3-glucoside) suppresses histamine release significantly better than kaempferol, having a histamine release suppression action about the same as that of epinephrine of 150 times the concentration.

Experimental Example 3: Intake test using NC/Nga mice

NC/Nga mice are conventional grade animals, and atopic dermatitis model mice that spontaneously develop atopic dermatitis. Moreover, the development of atopy is accompanied by a rise in serum IgE level.

Ten 4-week-old NC/Nga male mice were purchased from Japan SLC, and were reared at a room temperature of 23 ± 3°C and a humidity of 55 ± 15%, with a 12 hour light-dark cycle (light period 7:00 to 19:00). The mice were kept 5

to a cage, and were fed a standard diet (Labo MR Stock, Nihon Nosan Kogyo K.K.) for a 7-day preliminary period, before being divided into a control group and a group to be administered astragalin, each of 5 mice. The mice were then allowed to eat the following diets ad libitum.

Control group: A diet prepared by adding α corn starch (0.0007 %, Oriental Enzyme K.K.) to MF powder (Oriental Enzyme K.K.) and then mixing in a mixer.

Astragalin-administered group: A diet prepared by adding astragalin (0.0007 %) to the MF powder and then mixing in a mixer.

It was observed with the naked eye whether or not the NC/Nga mice had developed atopic dermatitis at the start of the experiment (5 weeks old) and then 1-week intervals until the end of the experiment (13 weeks old). The following judgement criteria were used.

No dermal symptoms: 0

Slight inflammation or scratch wounds: 1

Medium degree inflammation, scratch wounds or bleeding: 2

Severe inflammation, scratch wounds or bleeding: 3

For each mouse, the judgement was carried out for each of the head, the shoulders and the back, and then the highest of the three scores was taken as the 'maximum score'. The results are shown in Fig. 3 as the mean values of the maximum score for the two groups. In the

control group, symptoms started to appear at 7 weeks old, 3 of the 5 mice had developed symptoms by 8 weeks old, and 4 of the 5 mice had developed symptoms by the end of the experiment (13 weeks old). In the astragalin-administered group, on the other hand, not one mouse had developed symptoms by the end of the experiment (13 weeks old).

The serum IgE level was measured at the start of the experiment and then at 2-week intervals until the end of the experiment by collecting blood from the orbit and then using a mouse IgE measurement kit 'Yamasa' EIA (lot 702). The serum IgE level measurement values were represented by the mean and standard deviation. The differences between the two groups were tested for statistical significance using t-tests, and a significance level of 5% or less was determined significant. The results are shown in Fig. 4.

In both of the groups, the serum IgE level started to rise at age 7 weeks and then rose gradually with age. At the end of the experiment (13 weeks), however, the serum IgE level was 6,018 ng/ml for the control group but only 1,225 ng/ml for the astragalin-administered group, with the difference between the two being statistically significant, showing that the astragalin had suppressed the rise in serum IgE level.

It was thus verified that administering astragalin suppresses both the incidence rate of atopic dermatitis

and the rise in serum IgE level.

Experimental Example 4: Expression of IL-4 mRNA using the RT-PCR

A human basophilic leukemia cell line (KU812) was cultured at 37°C with 5% CO_2 in an RPMI 1640 culture medium (Gibco) containing 10% bovine fetal serum (treated at 56°C for 30 minutes). The KU812 cells ($5x10^5$ cells/ml) were then stimulated using astragalin (0, 1.1, 3.3 or 11 μM) and an A23187 ionophore (1 μM). After washing, the total RNA was collected using RNAzol (Biotex, USA). 500 ng of the collected total RNA was mixed with an RT mixture (Perkin Elmer Cetus, USA), and then incubation was carried out for 5 minutes at 99°C followed by 60 minutes at 37°C. After the RT products had been obtained, PCR amplification was carried out using an IL-4-specific primer and a β-actin-specific primer. The products were subjected to migration in 2% agarose gel and stained with ethidium bromide, and then the amount of expression was evaluated. As a result, it was found that the IL-4 expression of the basophilic leukemia was suppressed through administration of astragalin, with there being particularly marked effects when the concentration was 3.3 μM or 11 μM.

IL-4 is a Th2 cytokine that is involved in IgE production. The fact that suppression of IL-4 expression

was observed thus shows that astragalin is involved in suppressing the rise in IgE level.

Experimental Example 5

Thirteen volunteers who had previously experienced pollinosis were asked to drink 240 μg of astragalin (in the form of 1ml of concentrated persimmon leaf extract) dissolved in a suitable amount (50 to 200 ml) of water or hot water twice a day (morning and evening) starting 14 days before the start of the pollen season and ending 14 days after the start of the pollen season.

Sneezing, nasal discharge, nasal congestion and extent of impediment to daily life were evaluated using a points system both during the 7 days immediately before stopping drinking the astragalin solution ('while drinking' below) and during the 7 days immediately after stopping drinking the astragalin solution ('after stopping drinking' below).

For sneezing, 1 point was recorded for each sneeze. For nasal discharge, 1 point was recorded each time the nose was blown. For nasal congestion, 3 points were recorded when the nose was completely blocked such that breathing through the nose was impossible, 2 points were recorded when the nose was blocked such that breathing through the nose was difficult, 1 point was recorded when

the nose was slightly blocked, and 0 points were recorded when the nose was not blocked. For extent of impediment to daily life, 3 points were recorded when one could not settle down to work at all, 2 points were recorded when there was some impediment to working, 1 point was recorded when there was little impediment to working, and 0 points were recorded when there was no impediment to working; points were recorded daily.

The total point scores for the 7-day 'while drinking' period and the 7-day 'after stopping drinking' period are shown in Table 1 below as mean values over the 13 volunteers.

Table 1

	Sneezing	Nasal discharge	Nasal congestion	Impediment to daily life
While drinking	23±21	21±26	3±3	1±2
After stopping drinking	68±79	62±83	13±11	10±10
p value	0.001	0.003	0.003	0.005

It can be seen that the point score for each of the symptoms increased after stopping drinking the astragalin solution compared with while drinking the astragalin solution. Various symptoms of pollinosis can thus be expected to be alleviated by ingesting astragalin.

Two of the volunteers had a rough skin condition at the time of starting to drink the astragalin solution, but the condition improved while drinking the astragalin solution. Rough skin conditions can also be expected to be improved upon applying astragalin to the skin in the form of a cosmetic.

Formulation examples are given below. Each of the formulations can be prepared following conventional methods for the form to be made.

Formulation Example 1: Chewable tablet

	(mg)
Astragalin	5
Xylitol	300
Aspartame	4
Magnesium stearate	10
Aromatic	1

Formulation Example 2: Chewing gum

	(g)
Gum base	20
Powdered sugar	60.5
Starch syrup	18
Aromatic	1
Astragalin	0.5
Total	100

Formulation Example 3: Ice cream

	(g)
Concentrated milk	30
Fresh cream	30
Sugar	18
Emulsifier	0.3
Stabilizer	0.5
Aromatic	0.3
Egg extract	1
Astragalin	0.5
Water	19.4
Total	100

Formulation Example 4: Chocolate

	(g)
Cacao mass	22
Whole milk powder	10
Cacao butter	19.9
Lactose	5
Sugar	40
Aromatic	0.1
Egg extract	1
Astragalin	2
Total	100

CLAIMS

1. A composition for preventing or treating type I allergy and diseases associated with type I allergy, comprising kaempferol-3-glucoside as an active ingredient.

2. The composition according to claim 1, wherein the composition is a food composition for preventing type I allergy and diseases associated with type I allergy.

3. The composition according to claim 1, wherein the composition is a pharmaceutical composition for preventing or treating type I allergy and diseases associated with type I allergy.

4. The composition according to claim 1, wherein the composition is an external preparation for skin for preventing or treating type I allergy and diseases associated with type I allergy.

5. The composition according to claim 1, wherein the diseases associated with type I allergy are atopic diseases.

6. The composition according to claim 1, wherein

the disease associated with type I allergy is pollinosis.

7. A method for preventing or treating type I allergy and diseases associated with type I allergy by ingesting or administering an effective amount of kaempferol-3-glucoside.

8. The method according to claim 7, wherein the diseases associated with type I allergy are atopic diseases.

9. The method according to claim 7, wherein the disease associated with type I allergy is pollinosis.

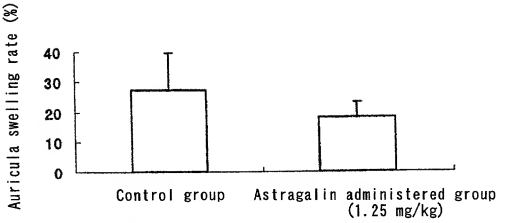

Figure 1

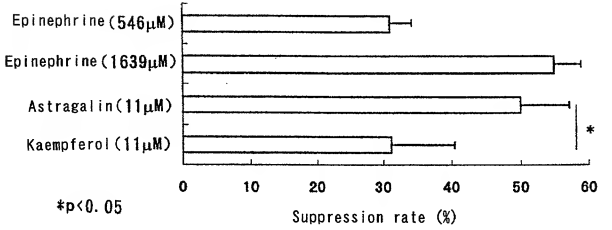

Figure 2

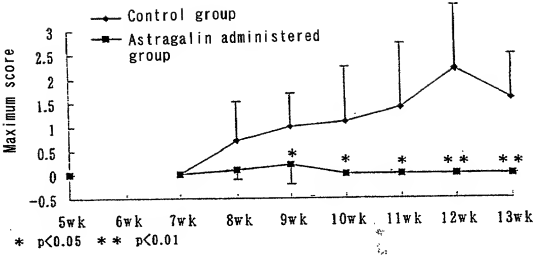

Figure 3

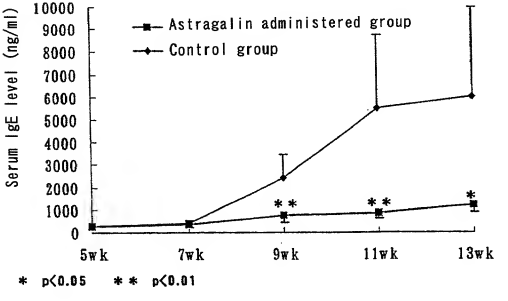

Figure 4

DECLARATION AND POWER OF ATTORNEY - USA PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled COMPOSITIONS FOR PREVENTING AND TREATING TYPE I ALLERGY

the specification of which:

(a) ☐ is attached hereto; or

(b) ☒ Initial documents for entry into the U.S. National Phase was filed on September 21, 2001 as Application No. 09/937365 or Express Mail No., as Application No. not yet known and was amended on (if applicable); or

(c) ☒ was described and claimed in PCT International Application No. PCT/IP00/01801 filed on March 24, 2000 and as amended under PCT Article 19 on (if any) and/or under PCT Article 34 on (if any).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56;

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent, design or inventor's certificate or any PCT international application(s) listed below and have also identified below any foreign application(s) for patent, design or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed for the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 U.S.C. § 119
Japan	1999-84395	26/03/1999	☒ YES NO ☐
Japan	1999-123633	30/04/1999	☒ YES NO ☐
Japan	1999-173731	21/06/1999	☒ YES NO ☐
			☐ YES NO ☐

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below, and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application:

United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Prior U.S.A. Application(s)

Application No.: _____ Filing Date: _____ Status: _____

POWER OF ATTORNEY: I hereby appoint the registrants of Knobbe, Martens, Olson & Bear, LLP, 620 Newport Center Drive, Sixteenth Floor, Newport Beach, California 92660, Telephone (949) 760-0404, Customer No. 20,995.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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